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X-RAY SMALL ANGLE MEASUREMENTS ON 1% SOLUTION OF HAEMOGLOBIN CORPUSCULAR PROTEIN

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INTRODUCTION

ABSTRACT. The small angle scattering due to 1% solution of haemoglobin in 0.85% solution of NaCl has been investigated. It has been observed that the scattering curve in the region from 600 Å to about 900 Å is fairly horizontal showing the absence of interparticular interference and molecular association

A sample of 1% solution of haemoglobin in 0.85% solution of NaCl from Behring Works, Germany (Handels' product), was investigated with the help of the well-known small angle scattering camera of Prof. Kratky (1958) in order to find out whether any remarkable feature is observable in the innermost portion of the scattering curve. In the case of interparticular interference this part of the curve will slope down, whereas in the case of molecular association the slope will be of the opposite nature.

EXPERIMENT

The experimental arrangement consisted of the well-known small angle scattering camera of Prof. Kratky fitted with a Geiger Muller counter tube together with the beam measuring apparatus of the firm Seimens and Halske. The source of X-radiation was a standard Phillips unit with a tube of copper anticathode. Before proceeding with the measurements on haemoglobin the Seimens apparatus was tested for its stability by taking a standard solution of colloidal gold as the scatterer. The beam-intensity was kept constant by stabilising the primary voltage supplied to the X-ray unit. Keeping the deffracting angle constant the intensity of the scattered ray was measured by using a nickel filter. By registering 6000 impulses each time over consecutive intervals of time it was found that the experimental fluctuation of intensity was in the neighbourhood of the statistical value $100/\sqrt{6000}$ in percentage, thus proving the stability of the apparatus. The natural counts due to cosmic rays were very much reduced by surrounding the counter tube with a very thick cylinder of lead. In order to prove the accuracy of the whole arrangement the full scattering curve of colloidal

gold in mandol oil was obtained by the filter difference method. This curve was compared with the curves, after reduction to proper scale, obtained by anti-coincidence measurements of M. Bishop and by photographic-photometer measurements of Dr. Krutz. The superposition of the later two curves with the one obtained in the present procedure showed very good agreement among them thus providing the accuracy of the adjustment. Measurement of the sample 1% solution of haemoglobin in NaCl was now undertaken. The blind curve with 0.85% solution of NaCl in a mark capillary of 0.75 mm diameter was first obtained by using Co and Ni filters. Then the same capillary was used as the container for 1% solution of haemoglobin in 0.85% solution of NaCl and the curve due to the sample was obtained. The difference-curve gives the scattering due to the pure sample itself. As haemoglobin is very sensitive all arrangements were made to keep the temperature of the sample constant throughout the measurements by using a good refrigerator for cooling the whole chamber. A fresh solution from Behring Works Germany was thus examined.

RESULTS AND DISCUSSION

The blind measurements due to 0.85% solution of NaCl are given in Table I, and the corresponding curve B is shown in figure 1. Table II gives the measurements due to 1% solution of haemoglobin in 0.85% solution of NaCl, the relative curve is marked as P in the above figure. Curve D is the difference curve i.e., the scattering due to the protein molecules themselves. It is evident from figure

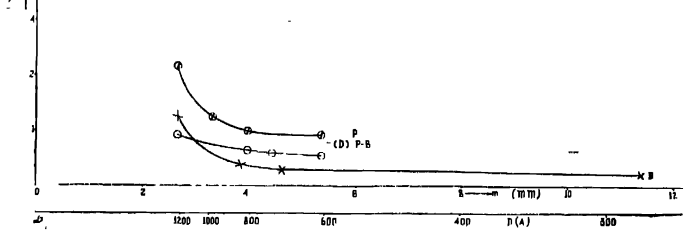

Figure 1.

1 that the difference curve runs horizontal from about 900 Å to 600 Å. The true nature of the scattering curve with low concentration is therefore purely due to particle-scattering and is in good agreement with the theory of Debye (1915). Thus there is no interparticular interference nor any molecular association. A very small rise in the scattering curve at about 1200 Å is probably due to small

amounts of association. It is rather very difficult to ascertain the exact nature of the curve in the close vicinity of 1200 Å (Kratky, 1958) as this is the lowest approachable limit of the improved types of cameras. Further measurements are necessary to check up the curve in this region

TABLE I

Blind measurements for 1% haemoglobin in 0.85% solution of NaCl.

Z_H	$m(mm)$	I	t	I/t	Ni-Co	Filter
441	1 16	837	900	0 93	0 33	
371	0.46	927	900	1 03	0.35	
361	0 36	1000	900	1.12	0 46	Ni
352	0 27	2162	900	2 40	1 24	
441	1 16	539	900	0.60		
371	0.46	612	900	0 68		
361	0 36	594	900	0 66		Co
352	0.27	1044	900	1 16		

S_1 5/100 mm. S_1' 10 mm S = 7/100 mm S' = 8 mm. Z_{H_0} 328.5

TABLE II

Sample, 1% Haemoglobin in 0.85% NaCl

Z_H	$m(mm)$	I	t	I/t	a.R.	Ni-Co	Remark*
352	0 27	2.10^4	5290	3.78		2.21	
358	0.33	$2\ 10^4$	8380	2 39		1.18	Ni
365	0 40	$1\ 10^4$	4566	2 19		1.01	
361	0.40	1.5×10^4	3966	3 78		...	S = 15/100
375	0.54	$1\ 10^4$	2793	3.58	2.071	0 97	F = 0 579
352	0.27	2.10^4	12732	1.57			Co
358	0.33	2.10^4	16581	1.21			
365	0.40	1.10^4	8475	1.18			
361	0.40	1.5×10^4	8502	1.76	..	....	S = 15/100
375	0 54	$1\ 10^4$	6080	1.64	1 10		F = 0.6701

* Filter, Slit width, Reduction factor (F). a.R. = after reduction.

S_1 = 5/100 mm S_1' = 10 mm. S = 7/100 mm. S' = 8 mm. Z_{H_0} = 328.5.

$$m = Z_H - Z_{H0} + S/2$$

m is measured in mm. It is the height of the center of the counter window from the center of the direct beam.

$$m = \chi a/D$$

a — distance of the film or the counter tube from the scatterer.

χ = wave length.

D = Bragg's value in Å.

Z_H = Height of the counter tube.

Z_{H0} — Zero position of the counter tube i.e., the center of the primary beam.

S = Width of the slit of the counter window

S' = Length of the slit of the counter window.

S_1 = Width of the entrance slit.

S'_1 — Length of the entrance slit

$\tilde{I}$ = Verschmierte intensity measured in impulses, (Intensity not corrected for collimation error.)

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